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(19)



(54) PHARMACEUTICAL AND FOOD FORMULATIONS

(71) We, THE WELLCOME FOUNDATION LIMITED, of 183-193 Euston Road, London, N.W.1. a company incorporated in England, HANS OLAF BANG of Klostermarken 47, 9000 Aalborg Denmark, and JØRN DYERBERG of Engholmvej 9, 9200 Aalborg SV. Denmark both Danish subjects do hereby declare that the invention for which we pray that a Patent may be granted to us and the method by which it is performed, to be particularly described in and by the following statement:

The present invention relates to the treatment or prophylaxis of thrombo-embolic conditions.

Although it is known that many substances can affect platelet aggregation, it cannot be predicted, from a knowledge of the effect of a particular substance on aggregation of platelets, whether or not the substance will have an inhibitory or stimulatory (or neutral) effect on thrombus formation *in vivo*. This is largely because it is not known what initiates formation of a thrombus or embolus in, for example, strokes or myocardial infarction. As an example of this unpredictability, aspirin is a good inhibitor of platelet aggregation *in vitro* and *in vivo*, but it is not an anti-thrombotic agent, in particular it cannot disperse a preformed thrombus.

M.J. Silver, J.B. Smith, *et al.*, (Prosta-glandins Dec. 1973, Vol. 4, No. 6, pages 863 to 875) showed *in vitro* that many compounds can influence the platelet aggregating effects produced by the dietary component arachidonic acid (5,8,11,14-eicosatetraenoic acid, alternatively C20:4; n-6 acid i.e. a fatty acid containing 20 carbon atoms having 4 double bonds, the one at the highest numbered position being at a position 6 bonds from the end of the molecule remote from the carboxyl group, and n being the number of carbon atoms in the straight chain). These *in vitro* tests in human citrated platelet rich plasma, cannot be unambiguously related to *in vivo* behaviour in a thrombus formation-prone mammal, including man. M.J. Silver *et al.* found in their tests that the platelet aggregation induced by arachidonic acid, as sodium arachidonate, can be inhibited by many materials including adenosine; β -naphthol; non-steroidal, anti-inflammatory agents, such as indomethacin, sodium salicylate and aspirin; unsaturated fatty acids, such as 11, 14, 17-eicosatrienoic acid; 8,11,14-eicosatrienoic acid (dihomo- γ -linolenic acid, DHLA); 5,8,11,14,17-eicosapentaenoic acid; 5,8,11,14-eicosatetraenoic acid; and 4,7,10,13,16,19-docosahexaenoic acid; and human albumin. They also found that the platelet aggregation induced by collagen and a second wave of platelet aggregation induced by adenosine diphosphate (ADP) could be inhibited by β -naphthol, aspirin, 8,11,14-eicosatrienoic acid, 5,8,11,14,17-eicosapentaenoic acid and human albumin. Silver *et al.* further found that various fatty acids on their own did not induce platelet aggregation. The acids they mentioned were 8,11,14-eicosatrienoic acid; 11,14,17-eicosatrienoic acid; 5,8,11,14,17-eicosapentaenoic acid; 5,8,11,14-eicosatetraenoic acid; 4,7,10,13,16,19-docosahexaenoic acid; linolenic acid; linoleic acid; oleic acid; arachidic acid; stearic acid; and decanoic acid.

Silver *et al.* appear to conclude that arachidonic acid has an important place in hemostasis and thrombosis, and that its effects can be inhibited *in vitro* by various compounds, particularly albumin. They suggested that albumin may be an important controlling factor in hemostasis and that the ability of albumin to bind arachidonic acid in circulating blood might be the way it inhibits the effect of arachidonic acid. They further suggested this binding capacity may depend on, for example, the availability of binding sites and the competition between arachidonic acid and other fatty acids and other classes of substances.

Presumably, therefore, the more competitive substances available, the more free arachidonic acid would be available and the more likely platelet aggregation would be and so, if these phenomena were to be related, the more likely thrombus formation would be.

Various attempts have been made to investigate in man the effects of various fatty acids on diseases involving thrombus formation, but without any clear conclusions emerging.

For example, the Norwegian Vegetable Oil Experiment of 1965-66 was carried out before the work of Silver *et al.* and was reported by H. Natvig, Chr. F. Borchgrevink, *et al* in Scand. J. Clin. Lab. Invest. 22. Suppl. 105, 1-20, (1968). The study compared the effects on human mortality rates caused by various coronary heart diseases, including myocardial infarction, of two diets, one containing sunflower seed oil (about 63% of linoleic acid) and the other containing linseed oil (about 55% of linolenic acid); 10 ml. of either oil be taken per day. The group taking the more highly unsaturated linolenic acid was found to be more at risk than the group taking the linoleic acid.

Linoleic acid, and, in rats, eicosapentaenoic and docosahexaenoic acids are known to decrease blood plasma cholesterol levels, which are believed to be connected with atherosclerosis. Atherosclerosis is often found in persons who have suffered from a myocardial infarct. However, there appears to be no causal relationship, because Robertson (Lancet, (1959), i, 44) found that in Jamaica, although extensive atherosclerosis is regularly found in the native population at necropsy, it is very seldom associated with secondary thrombi or with myocardial infarction. Further, myocardial infarcts can occur in the absence of highly developed atherosclerosis.

Yet another possible dietary factor that has been suggested (P.B. Kernoff, A.L. Willis, K.J. Stone, J.A. Davis and G.P. McNicol, British Med. J., 1977, 2, 1441-1444) as helping to inhibit thrombosis is DHLA. DHLA is a biosynthetic precursor of prostaglandin E₁ (PGE₁), which is a powerful inhibitor of platelet function, and is said to be attractive as an antithrombotic agent. It was found that there was, as hoped, a rise (mean 55%) in production of the desirable PGE₁ but in six men out of the eight tested there was also a rise (mean 33%) in production of the undesirable prostaglandin E₂ (PGE₂). Furthermore, these results were not clearly dose related. There was also a lowering of heparin-neutralising activity of plasma and this activity has been found to be high in thrombotic states. However, the authors did not know the extent to which heparin-neutralising activity reflects basic pathological mechanisms, and so its relationship with thrombosis was unclear.

The authors of the paper speculated that "Perhaps small doses of DHLA may be equally if not more effective than major dietary manipulations in preventing and treating these conditions" i.e. atherosclerosis and coronary heart disease. However, the author of an editorial in the same edition of the Journal (pages 1437 and 1438) was more cautious and thought that "Trials of agents and regimens that modify the platelet prostaglandin mechanisms must be carried out before we can tell whether the results obtained by McNicol and his colleagues have any clinical application". The reasons for his caution lay in the ignorance that exists of the mechanisms involved *in vivo* in thrombotic situations, when investigative tests have only been carried out on shed blood.

This at least partially attractive work with DHLA throws some doubt on the frequently quoted view that highly unsaturated fatty acids in the diet are more beneficial than their more saturated analogues, especially as the even less saturated linoleic and linolenic acids can be metabolised to DHLA. This doubt is strengthened by the fact that arachidonic acid which is undesirable (see Silver *et al* and Kernoff *et al* above) is even more unsaturated (4 carbon-carbon double bonds) than DHLA (three carbon-carbon double bonds).

We have now surprisingly found that among the many fatty acids (all Z)-5,8,11,14,17-eicosapentaenoic acid or its salts, esters or amides can be used to treat effectively, or provide effective prophylaxis against, thrombo-embolic conditions, hereinafter referred to simply as thrombosis. Examples of conditions where our findings may be useful are in the treatment or prophylaxis of cardiovascular disease mediated by the formation of a thrombus or thrombi, for example, myocardial infarction, strokes; or deep vein thrombosis during surgical operations.

We have found that (all Z)-5,8,11,14,17-eicosapentaenoic acid (hereinafter referred to simply as eicosapentaenoic acid) when injected intravenously into rabbits increases their bleeding time, thus demonstrating a decrease in the tendency of the blood to produce thrombi or adhere to damaged tissue. When infused into rabbit lung, eicosapentaenoic acid gives rise to a substance which has a powerful anti-aggregatory action on blood platelets. Eicosapentaenoic acid also has the unusual and important ability to disperse already formed thrombi.

For example, blood from an anaesthetised rabbit was allowed to drip over a continuously weighed collagen strip taken from the Achilles tendon of another rabbit. As the blood flowed over the strip, platelets and other cells adhered to it to form a thrombus until there was no further gain in weight of the strip. The blood was returned to the first rabbit under

gravity. When eicosapentaenoic acid was infused into the blood passing over the loaded strip a decrease in weight was observed, showing that at least a part of the aggregated platelets and other cells had been disaggregated from the loaded strip. This ability of eicosapentaenoic acid to bring about disaggregation of thrombus is important in the treatment of thrombosis, and also in its prophylaxis.

We have also found that human platelets when pre-incubated with eicosapentaenoic acid and then incubated with arachidonic acid and stimulated with ADP, aggregate less readily than when the pre-incubation is carried out with arachidonic acid. This suggested to us that, if human platelets could be 'primed' with eicosapentaenoic acid, they would be less susceptible to ADP stimulation and so less liable to form thrombi.

The dose of eicosapentaenoic acid needed for therapeutic or prophylactic effect will vary with the route of administration and the nature of the condition being treated, but will generally be at least 1 gram (g), preferably from 1.5 to 3g. per day. This is the dose for an average 70kg. man and the dose for other men or animals will vary *pro rata* according to their weight, i.e. about 20 to 40mg/kg.

Eicosapentaenoic acid is known to be present in cod liver oil and in other oils, e.g. menhaden oil, from which it may be extracted by methods known in the art or described in the literature. The eicosapentaenoic acid may also be synthesised by conventional methods of synthetic organic chemistry. The route chosen will depend on the availability of suitable starting materials. In practice the route used will depend on the relative costs of the various routes available to provide eicosapentaenoic acid of the right quality of human medical or veterinary use.

The amounts of eicosapentaenoic acid in naturally occurring or readily extractable materials such as cod liver oil or menhaden oil are such that it would not be possible to obtain the desired amount of eicosapentaenoic acid by administering them without also administering too many calories in the form of other fatty acids. Furthermore, as cod liver oil (and other fish oils) is rich in vitamin A (at least 850 international units (I.U.) per gram) and vitamin D (at least 851.U. per gram) administering enough cod liver oil to give the necessary amount of eicosapentaenoic acid would administer amounts of these vitamins greatly exceeding the recommended daily dose for humans and would lead to hypervitaminosis. The recommended daily dose is 5000 I.U. for vitamin A and 400 I.U. for vitamin D in humans. In the U.S.A. the Food and Drugs Administration has laid down that the daily intake of vitamin A should not exceed 10,000 I.U. and of vitamin D should not exceed 400 I.U. Amounts above this require a doctor's prescription. Accordingly if the eicosapentaenoic acid is to be administered without substantial modification of the recipient's diet (or at all), the acid used must represent at least 50% by weight, advantageously at least 90%, preferably at least 95% or all, by weight of the fatty acid content of the administered material. A pharmaceutically acceptable salt, ester or amide derivative of eicosapentaenoic acid may be used in the formulations, if desired, in which case the amount of the derivative is calculated as the corresponding amount of the parent acid in regard to the foregoing percentages. Arachidonic acid should preferably be absent or at most should be no more than 5% by weight of the fatty acid content. For example, a suitable quality of eicosapentaenoic acid comprises at least 90% by weight of the acid, about 2% by weight of each of arachidonic and dihomogamma-linolenic acids, the balance being other pharmaceutically acceptable fatty acids e.g. palmitic and oleic acids. If vitamins are present, as they may be, they should preferably not be present in amounts that would lead to their recommended daily intake being exceeded.

By administering the eicosapentaenoic acid at at least 90% of the fatty acid content, it should be possible to avoid substantial alteration of the diet of the recipient, except perhaps to reduce slightly the calorific content of the diet to allow for the extra calories from the eicosapentaenoic acid. However, if preferred, it may be possible to administer the eicosapentaenoic acid by replacing, say, butter and/or ordinary margarine by a special margarine, e.g. of the emulsion type, formulated so that in normal usage the recipient would receive the required amount of the eicosapentaenoic acid.

The eicosapentaenoic acid need not be administered as the acid itself but may be used as its pharmaceutically acceptable salts, esters or amides (which would be measured as their acid equivalents). Esters or amides which can be converted *in vivo* to the acid and other pharmaceutically acceptable products may be used, the preferred ester being the ethyl ester, but the methyl ester could perhaps also be used. The ester used is preferably not the cholesteryl ester as this would lead to some cholesterol being liberated, which may lead to an increase in the serum cholesterol level. The preferred salts are the sodium or potassium salts or any other pharmaceutically acceptable solid salt, as these are suitable for making into tablets. As eicosapentaenoic acid is highly unsaturated, it and its derivatives are readily oxidisable and formulations containing them should also contain anti-oxidants such as butylated hydroxy toluene, butylated hydroxy anisole, propyl gallate, a pharmaceutically

acceptable quinone and α -tocopherol.

Although it is preferred to administer the eicosapentaenoic acid (or its salts, esters or amides) (active compound) orally as this is a convenient route for routine administration, the active compound may be administered by any route by which it may be successfully absorbed, e.g. parenterally (i.e. subcutaneously, intramuscularly or intravenously), rectally or, in the case of women, vaginally.

While it is possible for the active compound to be administered as such or as a simple mixture of components, it is preferable to present it as a pharmaceutical formulation. The formulations, both for veterinary and for human medical use, of the present invention comprise the active compound as above defined, together with one or more acceptable carriers therefor and optionally other therapeutic ingredients. The carrier(s) must be 'acceptable' in the sense of being compatible with the other ingredients of the formulations and not deleterious to the recipient thereof. Formulations which contain eicosapentaenoic acid itself are preferably non-aqueous. Unit doses, e.g. tablets or capsules, of a formulation generally contain between 0.25 and 1.0 g, e.g. 0.5 g, of the active compound. Generally three doses would be administered per day.

Formulations include those suitable for oral, rectal, vaginal, intrapulmonary or parenteral (including subcutaneous, intramuscular and intravenous) administration.

As eicosapentaenoic acid itself is a liquid and tends to be unpalatable, it is preferably administered per orally in a capsule, for example one of soft gelatin, so that the eicosapentaenoic acid is not tasted. The capsule would generally be of a size to permit the required dose of eicosapentaenoic acid to be administerable in one or two capsules at each dose taking and so a capsule would be generally about 0.5 ml in size. Another way of disguising the taste of the acid is to formulate it as an emulsion to be taken orally. The acid could also be formulated to be spontaneously emulsifiable on being taken orally or being diluted before administration. An emulsion could also be of the multiple type e.g. the acid could be made into an oil-in-water emulsion with a pharmaceutically acceptable surface active agent and then this emulsion could be emulsified in another oil, e.g. arachis oil. Alternatively, the acid could be similarly formulated into a water-in-oil emulsion and then this emulsion itself emulsified in water. The various types of emulsion could be presented as an oral gel or as a stiff emulsion, such as an emulsion margarine. Other methods of disguising the taste are to absorb the acid onto a carrier or carriers such as kaolin, chalk, calcium phosphate, calcium sulphate, starch, a micro-crystalline cellulose or methyl or other modified cellulose. The resulting powder could be sold as such or flavoured, and perhaps made into tablets or capsules, each tablet or capsule containing, for example, about 0.5 g of eicosapentaenoic acid as such or in the form of a solid derivative. Tablets could be film- or sugar-coated.

As for the salts, e.g. the sodium or potassium salts, these also tend to be unpalatable and tablets containing them, and representing for example 0.5 g of acid, should preferably be coated e.g. by film or sugar. Other method of oral administration, e.g. cachet or lozenge, may also be used in appropriate circumstances. The esters or amides may be formulated as for the acid or the salts, depending on whether they are liquid or solid, respectively.

If desired an oral formulation can be presented as a sustained release formulation, for example as beads or micro-capsules in a capsule.

A formulation for intramuscular administration could be in the form of an emulsion. A formulation for intravenous injection could be in the form of a mixture that would spontaneously emulsify upon injection.

For rectal administration the acid or derivative could be formulated into a suppository in a triglyceride base e.g. cocoa butter, a Witepsol (registered Trade Mark) or Suppocire or placed in a soft gelatin suppository capsule.

The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing into association the active compound with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active compound with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product into the desired formulation. In the present specification and claims the term "carrier" includes one which is suitable for administration to a recipient and substantially encloses the active compound e.g. the body of a capsule or the coating on a coated tablet.

Accordingly, the present invention provides:-

(a) a pharmaceutical formulation comprising (all Z)-5,8,11,14,17-eicosapentaenoic acid or a pharmaceutically acceptable salt, ester or amide thereof, and a pharmaceutically acceptable carrier, at least 50% by weight, preferably at least 90% by weight of the fatty acid content of the formulation being (all Z)-5,8,11,14,17-eicosapentaenoic acid;

(b) a method of preparing a pharmaceutical formulation according to (a) comprising

bringing the components into association with one another;

(c) a margarine or butter formulation including (all -Z)-5,8,11-14,17-eicosapentaenoic acid or a salt, ester or amide thereof in an amount to provide at least 3% by weight of the eicosapentaenoic acid.

The present invention is illustrated by the following Examples.

EXAMPLE 1

Blood for human volunteers who had not taken aspirin for the previous two weeks was collected from an ante-cubital vein in sodium citrate (0.11M), 1 part of citrate to 9 parts of blood. Plasma was separated from the blood by centrifugation at 160g (5 minutes) as a platelet rich plasma (PRP).

Studies on platelets were performed with arachidonic acid (AA), eicosapentaenoic acid (EPA) prepared as potassium salts (see Schrör, K., Moncada, S., Ubatuba, F.B., and Vane, J.R., Eur. J. Pharmac., 1978, 47, 103) and with ADP or thrombin in a coagulation apparatus e.g. 'Fibromate' (Bie & Bernsted, Copenhagen, Denmark).

Aggregation was recorded both turbidimetrically and nephelometrically in a cylindrical cuvette, containing 300 μ l of PRP at 37°C and stirred magnetically at 800 rpm; alternatively a Payton dual channel aggregometer was used with 500 μ l PRP.

In contrast to AA, EPA did not induce aggregation in human PRP at concentrations of EPA (1.33, 2.66 and 5.3 mM) about 4 or more times greater than AA (0.33, 0.66 and 1.3 mM). At lower concentrations in the range of from 0.01 to 0.5 mM EPA somewhat inhibited platelet aggregation induced by ADP (2 μ M) in the human PRP.

The anti-aggregating effect of EPA (0.065 mM), however, was not due to its conversion by platelet cyclo-oxygenase because the anti-aggregating effect was present with aspirin-treated platelets which did not respond to AA (0.065 mM) but were aggregated by thrombin (0.04-0.4 U/ml), and the anti-aggregating effect was also present in first phase aggregation induced by ADP (2 to 5 μ M) in aspirin-treated platelets.

EXAMPLE 2

Vascular tissue (thoracic and abdominal aorta) was obtained from freshly killed rats. Approximately 100 mg of tissue was chopped and washed once in ice cold Tris buffer (0.05 M, pH 7.5). After testing its ability to inhibit thrombin-induced platelet aggregation when added to the platelet cuvette, the tissue was washed several times in 10 ml of ice cold Tris buffer to remove blood and adhering platelets. The tissue was then quickly frozen to -60°C, crushed to a coarse powder and resuspended in five volumes of Tris buffer. This suspension of vascular tissue was kept to ice during the experiments and used for incubation studies.

Blood was obtained from the ante-cubital vein of human volunteers that had taken aspirin (1.5g per day) for the 3 days before blood sampling. Washed human platelets were obtained from this blood as described by Vargafitig, B.B., Tranier, Y, and Chignard, M, (Prostaglandins, 1974, 8, 133). Aggregation tests were carried out as in Example 1.

To see if the suspension of vascular tissue could synthesise any material having an anti-aggregatory effect on human platelets, platelets were obtained as described above from volunteers who had taken aspirin, so that their platelets could not produce prostaglandin endoperoxides that could be utilized by the vascular tissue to make anti-aggregatory material. Moreover, washed platelets were used to avoid any possibility of the vascular tissue utilizing any AA in the plasma. Under these conditions anti-aggregating activity could be formed by the vascular tissue only from endogenous or exogenously added precursors.

The initial suspension of vascular tissue (10 to 50 μ l) described above inhibited aggregation induced by thrombin (0.04 to 0.4 U/ml). This inhibitory activity was abolished by repeated washing (5 to 20 times) of the tissue by centrifuging (30 seconds in an Eppendorff centrifuge), pouring off the supernatant and resuspending in fresh buffer (0.5 ml). The general of inhibitory activity against primary phase aggregation induced by ADP (2 to 5 μ M) or aggregation induced by thrombin (0.04 to 0.4 U/ml) could be restored by adding washed vascular tissue and EPA to the washed platelets from aspirin-treated volunteers. The generation of anti-aggregating activity was prevented by the pretreatment of the washed vascular tissue with indomethacin (5 to 10 μ g/ml). Thus, the vessel wall cyclo-oxygenase could utilise EPA to form anti-aggregating activity.

The anti-aggregating activity formed might have been due to displacement of endogenous AA by EPA and not to direct utilization of EPA. However, the same concentrations of DHLA incubated with washed vascular tissue did not lead to the formation of anti-aggregating material.

EXAMPLE 3***The Effect of Eicosapentaenoic Acid on Bleeding Time In the Rabbit***

- Four male New Zealand white rabbits (Ranch) weighing 2.0 to 2.5 kg were anaesthetized with sodium pentobarbitone (40 mg/kg). The marginal ear vein was cannulated for infusions (0.1 ml/min) of eicosapentaenoic acid. The potassium salt of eicosapentaenoic acid (95% pure and containing about 2% AA and 2% DHA, balance C-18 fatty acids) was dissolved in 50 mM Tris-HCl buffer pH 8.0 kept on ice and shielded from light. Infusions of either the Tris vehicle or eicosapentaenoic acid were made 5 minutes before and continuously during the measurement of bleeding time.
- The internal surface of the ear without the cannular was carefully shaved. The ear was transilluminated so that blood vessels were clearly visible. Cuts, approximately 0.4 cm long and deep enough to cause an upwelling of blood within 15 seconds, were made with a new scalpel blade in an area free of visible blood vessels and in a direction parallel to the nearest blood vessel. The cut was gently blotted every 15 seconds with filter paper (Whatman No.1).
- Bleeding time was measured to the nearest 15 seconds from the time of incision until dots of blood were no longer visible on the filter paper. If there was a plasma exudate from the cut, the end point was considered as the time when the exudate no longer had a reddish tinge. When bleeding time was longer than 10 minutes, the cut was then blotted every 30 seconds. The bleeding time at each dose was a mean of 3 estimations.
- Two rabbits were pretreated with aspirin 100 mg/kg i.v. injection 4 hours before the experiment. Two rabbits were given 0.5 ml Tris pH 7.5 in 4 ml saline in the same way to act as controls. The results obtained are set out in Tables 1 and 2.

TABLE 1*Controls i.e. no aspirin*

30	EPA Dose µg/kg/min	Bleeding Time*	
		Rabbit 1	Rabbit 2
35	0	3.5	3.0
	50		16.0
40	100	19.8	16.5
	200		**23.0

TABLE 2

Pretreated with aspirin 100 mg/kg i.v. injection 4 hours before test begun.

5	EPA Dose $\mu\text{g/kg/min}$	Bleeding Time* minutes		5
10		Rabbit 3	Rabbit 4	10
	0	5.3	4.7	
	100	7.3	6.3	15
15	200	4.5	**7.5	
20	* Mean of 3 estimations **Rate of infusion 0.2 ml/min. Accordingly when treated with aspirin, the rabbits showed little or no increase in bleeding time.			20
25	EXAMPLE 4 <i>Conversion of Eicosapentaenoic Acid in the Circulation of the Dog</i> Intravenous infusion of eicosapentaenoic acid (0.2 to $2 \text{ mg kg}^{-1} \text{ min}^{-1}$) caused systemic and pulmonary hypotension in chloralose anaesthetized dogs. Blood-bathed isolated strips of bovine coronary artery and rabbit coeliac artery are relaxed by the powerfully antiaggregatory material PGI_2 (5 to 10 ng/ml). When treated with antagonists of catecholamines and angiotensin II, these bioassay tissues, bathed in arterial blood, relaxed during infusion of eicosapentaenoic acid (0.6 to $2 \text{ mg kg}^{-1} \text{ min}^{-1}$, 2 dogs), equivalent to about 10 to 20 ng/ml PGI_2 at the highest rates. In one of these dogs after administration of indomethacin (5 mg/kg), subsequent infusion of eicosapentaenoic acid ($2 \text{ mg kg}^{-1} \text{ min}^{-1}$ for 10 min) still caused hypotension but did not release any detectable activity in the bioassay tissues.			25
30	EXAMPLE 5 <i>Disaggregating Effect of Eicosapentaenoic Acid in the Rabbit</i> Rabbits ($2-3 \text{ kg}$) were anaesthetized with pentobarbitone sodium 30 mg/kg and heparinized (2000 U/kg). A carotid artery was dissected and blood was exteriorized and delivered with a roller pump to superfuse a strip of collagen from the Achilles tendon of a different rabbit. As the blood flowed over the tendon strip, the strip increased in weight over a period of 35 min. up to a maximum of from 180 to 200 mg . Thereafter any decrease in weight was due to platelet disaggregation.			30
35	Eicosapentaenoic acid infused intravenously ($50-500 \mu\text{g/kg/min}$) in five rabbits induced small disaggregating effects (approximately 20 mg). This effect of eicosapentaenoic acid could be inhibited by pre-treating the rabbits with aspirin (150 mg/kg).			35
40	EXAMPLE 6 A soft gelatin capsule to contain about 0.5 ml was sterilised and then filled with a composition containing more than 90% by weight of EPA, about 2% by weight AA, about 2% by weight DHLA with the balance being palmitic and oleic acids. The capsule was then sealed.			40
45	The capsule used may be transparent or coloured, and may also be of the hard gelatin type or made of polymethyl methacrylate for example.			45
50				50
55				55

EXAMPLE 7

A tablet formulation comprised:-

5	Sodium eicosapentaenoate	281 mg	5
	Starch	62 mg	
	Lactose	250 mg	
10	Polyvinyl pyrrolidone	3.5 mg	10
	Magnesium Stearate	3.5 mg	
15	Butylated hydroxy toluene	2 ppm	15
	TOTAL	600 mg	

The tablet was coated with sugar, although other coating agents could be used.

20

EXAMPLE 8

The formulation described in Example 7 in untabletted powder form may be used to fill hard gelatin capsules with 600 mg of the formulation.

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EXAMPLE 9

About 250 g of a conventional soft margarine formulation was thoroughly mixed with 8 g of eicosapentaenoic acid until a smooth consistency was reached.

WHAT WE CLAIM IS:

- 30 1. A pharmaceutical formulation comprising (all-Z)-5,8,11,14,17-eicosapentaenoic acid, or a pharmaceutically acceptable salt, ester or amide thereof, and at least one pharmaceutically acceptable carrier, at least 50% by weight of the fatty acid content of the formulation being (all-Z)-5,8,11,14,17-eicosapentaenoic acid. 30
2. A formulation according to claim 1 in which at least 90% by weight of the fatty acid content of the formulation is (all-Z)-5,8,11,14,17-eicosapentaenoic acid. 35
3. A formulation according to claim 1 in which at least 95% by weight of the fatty acid content of the formulation is (all-Z)-5,8,11,14,17-eicosapentaenoic acid.
4. A formulation according to any of claims 1 to 3, in which the fatty acid content comprises, in addition to the said eicosapentaenoic acid, about 2% by weight of arachidonic acid and about 2% by weight of dihomog- γ -linolenic acid. 40
5. A formulation according to claim 1 in which the fatty acid content is all or substantially all (all-Z)-5,8,11,14,17-eicosapentaenoic acid.
6. A pharmaceutical formulation comprising (all-Z)-5,8,11,14,17-eicosapentaenoic acid, or a pharmaceutically acceptable salt, ester or amide thereof, and at least one pharmaceutically acceptable carrier, said formulation being substantially free of vitamins. 45
7. A formulation according to any one of the preceding claims in which the eicosapentaenoic acid is present in the form of its sodium or potassium salt.
8. A formulation according to any one of claims 1 to 6 in which the eicosapentaenoic acid is present as its ethyl ester.
9. A formulation according to any one of claims 1 to 6 in which the said eicosapentaenoic acid is used. 50
10. A formulation according to any one of the preceding claims including an antioxidant.
11. A formulation according to any one of the preceding claims including a flavouring agent. 55
12. A formulation according to any one of the preceding claims in which the carrier comprises a solid.
13. A formulation according to claim 12 in which the carrier is or includes a capsule enclosing the remainder of the formulation.
14. A formulation according to any one of claims 1 to 11 in which the carrier is liquid. 60
15. A formulation according to claim 14 in which the eicosapentaenoic acid, salt, ester or amide forms a disperse phase in the carrier liquid.
16. A formulation according to claim 14 or 15 in capsule form.
17. A formulation according to any one of claims 1 to 12 in tablet form.
- 65 18. A formulation according to any one of the preceding claims in a form adapted for 65

oral, parenteral, rectal, vaginal or intrapulmonary administration.

19. A formulation according to any one of the preceding claims in unit dosage form.

20. A formulation according to claim 19 containing 0.25 to 1.0 g of the said eicosapentaenoic acid.

5 21. A method of preparing a formulation according to any one of the preceding claims comprising bringing the components into association with one another. 5

22. A margarine or butter formulation including (all-Z)-5,8,11,14,17-eicosapentaenoic acid or a salt, ester or amide thereof in an amount to provide at least 3% by weight of the eicosapentaenoic acid.

10 23. A margarine formulation according to claim 22 in the form of an emulsion margarine. 10

24. A capsule substantially as hereinbefore described either in Example 6 or in Example 8.

15 25. A margarine formulation according to claim 22 substantially as hereinbefore described in Example 9. 15

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